BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES OF THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application of:
Minxue Zheng et al.

Confirmation No. 9085

Application Serial No. 10/667,191

Group Art Unit: 1637

Filing Date: September 15, 2003

Examiner: Suryaprabha Chundura

Title: DUAL PURPOSE PRIMERS AND PROBES FOR PROVIDING ENHANCED HYBRIDIZAATION ASSAYS BY DISRUPTION OF SECONDARY STRUCTURE FORMATION

REPLY BRIEF

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Commissioner for Patents
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Sir:

Responsive to the Examiner's Answer mailed from the USPTO on June 14, 2011, and the Examiner's Answer Supplement mailed from the USPTO on July 20, 2011, applicants respectfully request the Board's consideration of the arguments set forth in this Reply Brief. This paper is timely filed within two months of the June 14, 2011, mailing date of the Examiner's Answer.

A. INDEPENDENT CLAIMS 1 AND 32

At page 10 of the Examiner's Answer, the Examiner paraphrases independent claims 1 and 32 of the instant application. Because the Examiner's paraphrased version of the claims does not include all of the claim limitations, it is not an accurate representation of the subject matter being claimed. In this regard, applicants are taking this opportunity to request that this Honorable Board ignore the Examiner's paraphrasing of independent claims 1 and 32 on page 10 of the Examiner's Answer and rely instead on the actual claim language, which is being reproduced herein for the Board's convenience.

Independent claim 1 is directed to a dual-purpose primer for amplifying a target nucleotide sequence in a target molecule, wherein the target molecule has a secondary structure forming region and further wherein the target nucleotide sequence contains a site of interest proximal to or contained within the secondary structure forming region wherein the primer comprises: (a) a primer sequence complementary to a segment of the target nucleotide sequence other than the secondary structure forming region; and (b) a blocking sequence substantially complementary to a segment of the secondary structure forming region, wherein the blocking sequence disrupts formation of the unwanted secondary structure in an amplicon thereby enabling detection and amplification of the site of interest.

Independent claim 32 is directed to a hybridization probe comprising (a) a probe nucleotide sequence complementary to a first nucleotide sequence in a target molecule, and (b) a blocking sequence substantially complementary to a second nucleotide sequence located within a secondary structure formation in the target molecule, wherein the secondary structure formation interferes with hybridization of the probe nucleotide sequence to the first nucleotide sequence and further wherein hybridization of the blocking sequence with the second nucleotide sequence disrupts the secondary structure formation in the second nucleotide sequence such that the probe nucleotide sequence is able to hybridize to the first nucleotide sequence.

B. HONEYMAN ET AL. DO NOT ANTICIPATE THE CLAIMED INVENTION

At pages 10-13 of the Examiner's Answer, the Examiner attempts to justify the citation of Honeyman et al. as an anticipatory reference for independent claims 1 and 32 by providing the following four rebuttal arguments.

First, the Examiner states that "the claims recite in alternative that the target nucleotide sequence comprises a site of interest proximal to *or* within a secondary structure forming region (emphasis added by Examiner)" and "[t]hus the assertions drawn to the site of interest within the secondary structure region are unpersuasive" (Examiners Answer, pp. 10-11). The Examiner, however, provides no rationale or explanation as to why the applicants' assertions regarding the site of interest within the secondary structure forming region are unpersuasive and instead rests the arguments on nothing more than the conclusory statement of "unpersuasivness" set forth above. In this regard, applicants respectfully request that this Honorable Board not give this first "argument" of the Examiner any weight as it does not rise to the level of an actual argument.

Second, the Examiner contends that applicants' arguments in support of the claimed invention exceed the scope of the claims, but the Examiner provides no analysis to support this contention (Examiner's Answer, p. 11). Instead, the Examiner appears to present an argument that suggests that applicants' claims read on the Honeyman et al. primers and probes because they are not specific to the primers and probes recited in the specification. The Examiner's position on this issue is most assuredly wrong. As explained in detail in the Appeal Brief, the Honeyman et al. probe is very different form the claimed primers and probes for a number of reasons. One of those reasons is that unlike the claimed primers and probes, which disrupt secondary structure formation, the Honeyman et al. SSCP snapback primer (the GR1-SB primer) is designed to form secondary structures in order to slow migration of PCR products for the normal gene so that they may be distinguished from the faster migration of the linear PCR products for the mutated gene (the GF2 primer), that latter of which do not form any secondary structures. Another reason is that Honeyman et al. do not teach, suggest, or contemplate a blocking sequence in any of the primers taught therein, which is axiomatic since Honeyman et al. is not blocking anything; rather, as explained above and in the Appeal Brief, the Honeyman et al. GF2 primer is forming a linear amplification product and the GR1-SB snapback primer is forming a PCR product with secondary structures. In other words, neither the GF2 primer nor the GR1-SB primer is blocking secondary structures (Appeal Brief, pp. 8-10 and 19).

Third, the Examiner attempts to argue that applicants are importing primer/probe sequence limitations from the specification into the claims; however, the Examiner fails to identify the offending primer/probe sequence limitations that applicant is "allegedly" importing from the specification. Applicants have reviewed the Appeal Brief that was filed on March 29, 2011, and the primer/probe arguments set forth therein very clearly address only the claim limitations, specifically, a primer sequence complementary to a segment of a target nucleotide sequence other than a secondary structure forming region and a blocking sequence substantially complimentary to a segment of the secondary structure forming region, wherein the blocking sequence disrupts formation of the unwanted secondary structure in an amplicon (Appeal Brief, pp. 10-18). If the Examiner thinks that arguments directed to the foregoing are not directed to the limitations of the claimed invention, then an explanation explaining why this is the case is required; since the Examiner has not provided such an explanation, applicants respectfully request that this Honorable Board not give this third "argument" of the Examiner any weight.

Fourth, the Examiner takes the position that Honeyman et al. teach the claim limitations and that applicants assertions regarding specific sequences are unpersuasive since the claims are not "restricted to the sequences of primers/probes, instead the claims require a target sequence with a site of interest, a secondary structure forming region and a blocking sequence…as discussed in the rejection." (Examiner's Answer, p. 11) Applicants note that the Examiner's statement as set forth above does not lend itself to a ready understanding of what the Examiner is trying to convey. Nevertheless, a review of applicants arguments at pages 15-18 of the Appeal Brief, where the invention of independent claims 1 and 32 is distinguished over Honeyman et al., only references specific sequences from Honeyman et al., which is the reference that the Examiner is citing as being the same as the claimed invention. That the Examiner takes the position that the specific sequences of Honeyman et al. are not applicable to the anticipation rejection over Honeyman et al. is paradoxical and turns the Examiner's own rejection on its head.

Notwithstanding the foregoing, a review of the Examiner's rejection of claims 1 and 32 in the final Office Action of November 9, 2010, as well as in the Examiner's Answer (p. 5), finds the Examiner referencing the arguments rejecting dependent claim 26. In the rejection of claim 26, the Examiner discusses an "inherent" secondary structure forming region in a "hypothetical" target of the Examiner's own fabrication. Applicants provided a detailed rebuttal analysis of this rejection on pages 15-18 of the Appeal Brief (where the primers and probes of claims 1 and 32 were distinguished from the Honeyman et al. primers). Because the Examiner's Answer fails to provide any rebuttal arguments that actually address the many issues raised by applicant in the Appeal Brief, applicants have nothing new to provide as counter arguments, but take this opportunity to redirect this Honorable Board to applicants' detailed analysis of Honeyman et al. in the Appeal Brief. Applicants submit that a thorough review of the teachings of Honeyman et al. will draw the only possible conclusion that the primers and probes of Honeyman et al. are different from those of the claimed invention.

Turning to the Examiner's rebuttal arguments for the dependent claims, these rebuttal arguments also have serious flaws.

First, in the last paragraph of page 11, the Examiner discredits applicants' arguments distinguishing claim 4 over Honeyman et al. by stating that "Honeyman et al. teaches (sic) that

the primers flank a site of interest (mutant allele), which clearly teach that at least one terminus is hybridized closer to or proximal to the site of interest of the target sequence."

Applicants submit that Honeyman et al. do not teach that the primers disclosed therein flank a site of interest and actually teach the opposite. Directing the Board's attention to the Discussion of Honeyman et al., there, Honeyman et al. explain that in their initial attempts at preparing the forward snapback primer, they designed the primer to anneal a few bases away from the normal sequence flanking the mutation. This design resulted in preferential amplification of the mutant allele in heterozygous animals with only small amounts of the normal product. Honeyman et al. attributed this result to the 3′ end of the normal reverse strand snapping back on itself to form a hairpin structure and *reducing amplification efficiency of the normal product*. Honeyman et al. then goes on to explain that the self-primed hairpin arrangement, with subsequent DNA extension, presumably formed before the forward snapback primer could participate in the PCR. Honeyman et al. then go on to explain that the self-priming was not observed in other preliminary studies in which the snapback primer *was designed to anneal at least 70 bases from the normal sequence flanking the mutation under investigation*.

The foregoing explanation from the Honeyman et al. Discussion shows that the snapback primer of Honeyman et al. does ***not*** flank the site of interest. With regard to the other primers of Honeyman et al., Honeyman et al. provides no teaching or suggestion regarding where the priming of the canine dystrophin gene occurs.

As explained in the Appeal Brief (pp. 8-10 and 19-20), Honeyman et al. teach the following four primers: (i) the GF2 primer, which the Examiner equates with the claimed primers and probes (*see*, FOA, p. 4 and Examiner's Answer, p. 7); (ii) the GR1 primer, which is used with the GF2 primer for conventional SSCP analysis (Honeyman et al., p. 735, Materials and Methods); (iii) the GR1-SB snap-back primer; and (iv) the GR2 primer, which is used with the GR1-SB primer for the snapback SSCP analysis (Honeyman et al., p. 735, Materials and Methods). The following discussion will explain in further detail how Honeyman et al. uses these primers and why these primers are ***not*** the same as the claimed primers.

As noted in the Appeal Brief and p. 735 of Honeyman et al. (Materials and Methods), the GF2 and GR1 primers were used for conventional SSCP analysis to detect the mutant allele with the GF2 primer corresponding to basepairs 135-114 of the canine dystrophin gene on intron 6, and GR1 primer corresponding to basepairs 805-792 on exon 7. The first page of Honeyman et

al. (p. 734, col. 1) explain that the causative canine dystrophin gene mutation is a single base change within the 3′ splice site of intron 6 in the canine dystrophin gene, which results in a loss of exon 7 and disruption of the reading frame so that transcription of the dystrophin gene is prematurely truncated by an in-frame stop codon on exon 8. The top part of Figure 3 of Honeyman et al. shows the normal allele of the canine dystrophin gene and the 3′ splice site where the normal adenine residue is replaced with a guanosine residue in dogs with the mutation. The legend for Figure 3 explains that the lower case letters identify intron 6 and the upper case letters identify exon 7. As noted in the Materials and Methods of Honeyman et al., the GF2 primer, which the Examiner equates with the claimed primers, corresponds to base pairs 135-114 of intron 6 of the canine dystrophin gene and has the sequence: 5′-CTT AAG GAA TGA TCC GCA TGG G-3′. The complement of the GF2 sequence is: 3′-GAA TTG CTT ACG AGG CGT ACC C-5′. A review of Figure 3 does not show the complementary sequence of the GF2 primer and the legend for the top portion of Figure 3 notes that it shows "*a portion* of the sequence for the normal allele of the canine dystrophin gene" (emphasis added here) without indicating and/or identifying the base pairs in the figure. In view of the foregoing, it follows that the despite the Examiner's assertions on page 11 of the Examiner's Answer that the Honeyman et al. "primers flank a site of interest," Honeyman et al. provides no such teaching, suggestion, and/or evidence of same.

In addition to the foregoing, it must be noted that Honeyman et al. is ***not*** concerned with the identification of the mutant allele, what Honeyman et al. is concerned with is the snap-back primer taught therein, which is designed to identify the normal allele of the canine dystrophin gene. In this regard, Honeyman et al. has no reason to go into a detailed teaching of the methods, techniques, and sequences that are used to detect the mutant allele (since these are known) and instead, uses the AJVR paper to disclose the methods and techniques that were used to design the GR1-SB primer, which is used to detect the normal dystrophin gene allele. In this regard, applicants note that the bottom part of Figure 3 shows how the GR1-SB primer snaps back and forms a conformation change in normal dogs, which have a adenine at the 3′ splice site, and that the GR1-SB primer does not snap-back in dogs with the mutant guanosine SNP. Because the primer for the mutant gene is linear and the primer for the normal gene has a conformation change, the normal PCR product migrates slower in a gel than does the PCR product of the mutant gene.

Second, despite the Examiner identifying the GF2 primer as the same as the claimed primers and probes, the Examiner discusses the snapback loop of the GR1-SB primer as equivalent to the claimed non-hybridizing spacer sequence of claim 5, 9, and 12. In this regard, it is clear that the Examiner is citing the GF2 primer when convenient and the GR1-SB primer when convenient with no appreciation for the teachings in Honeyman et al. regarding the differences between these two primers or the fact that the two primers are used for two different purposes and are not interchangeable.

Third, the Examiner's rebuttal arguments to applicants' analysis of claims 13 and 14 applies the same flawed logic as the rebuttal arguments for claims 5, 9, and 12, namely, the Examiner equates characteristics from the GR1-SB snap-back primer with the claimed primers, rather than characteristics of the GF2 primer, which the Examiner originally provided was the same as the claimed primers. In this regard, the Examiner's assertion on page 12 of the Examiner's Answer that the "reduction in the amplification efficiency of the normal product is attributable to the formation of hairpin (sic) structure because of the presence of the mutant allele, which indicates that the transcription is arrested because of the mutant nucleotide, which is considered a linker" is not an accurate statement and shows an inherent misunderstanding of the teachings of Honeyman et al.

As explained in the Appeal Brief and further herein, the GR1-SB primer snap-backs *only when it is primed to the normal canine dystrophin gene*; the formation of the conformation change as a result of the snap-back results in *reduced migration of the normal gene in a gel*, ***not*** in a reduction of amplification efficiency. Further, the only discussion in Honeyman et al. to reduced amplification efficiency is the discussion set forth above from the Discussion section of Honeyman et al. where Honeyman et al. explain how the initial attempts at designing the GR1-SB primer resulted in reduced amplification efficiency when the primer was annealed at a site flanking the site of interest. As noted above, modification of the priming site at least 70 base pairs downstream from the site of interest increased the amplification efficiency of the GR1-SB primer to the normal canine dystrophin gene. As is clear from the foregoing, Honeyman et al. developed the GR1-SB snap-back primer in order to ***correct*** the unsatisfactory amplification efficiency of the normal product *with traditional SSCP primers*, such as the original GF1 primer.

Further, the Examiner's assertion that the "mutant allele" (applicants are assuming that the Examiner is referring to the SNP site of the GR1-SB primer) is the same as the transcription

arresting linker of claims 13 and 14 is not supported by Honeyman et al. There is no teaching or suggestion anywhere in Honeyman et al. that the SNP site of the GR1-SB snapback primer halts transcription. Indeed, all that Honeyman et al. teach with respect to the mutant allele is that when it is present, the GR1-SB primer does not snap back and when it is not present, the GR1-SB primer does snap back. Any additional interpretation beyond these teachings is outside the scope of Honeyman et al. and requires additional evidentiary support.

Turning to the issue of the Examiner's claim construction analysis (Examiner's Answer, p. 12), applicants note for the record that in this section of the Examiner's Answer, the Examiner asserts that interpretation of the claims based upon the target is a necessary requirement of the claim constructions. Applicants submit that this statement is in direct contravention to the Examiner's statement on page 11 of the Examiner's Answer where it is provided that the target sequence exceeds the scope of the claims, which is directed to primers and probes. In other words, at page 11 the Examiner taking the position that the claims are directed only to primers and probes and that any claim interpretation ***by applicant*** that takes into consideration the target is outside the scope of the claims and at page 12, the Examiner undertakes a claim interpretation that concentrates exclusively on the target and takes the position that this claim interpretation is appropriate and proper. Applicants are sure that the Board appreciates the inequities associated with the Examiner's contradictory statements; indeed, it would be quite disturbing if the USPTO adopted a double-standard in claim interpretation where an Examiner is allowed to interpret claims with limitations that are prohibited to the applicant.

Notwithstanding the foregoing, applicants note for the record that page 24 of the Appeal Brief, which the Examiner is referencing in the last paragraph of page 12 of the Examiner's Answer, is ***not*** interpreting the claimed invention with regard to the target; rather, that page of the Appeal Brief specifically references the Examiner's failure to consider the ***blocking sequence*** in the prior Appeal (Appeal No. 2009-007969 Decided February 23, 2010) and notes that the Examiner's "functional" interpretation of the blocking sequence in the Final Office Action of November 9, 2010, is no different from the Examiner's prior act of completely ignoring the blocking sequence. Indeed, the Examiner's notable failure to reference and/or discuss the blocking sequence in the Examiner's rebuttal arguments in the Examiner's Answer (Examiner's Answer, pp. 10-13) appears to support applicants' position that the Examiner is continuing to ignore and/or disregard this important claim limitation.

Lastly, applicants note one more time for the record that the teachings of Honeyman et al. are comparable to the teachings of Wilton et al., which the Board previously held do ***not*** anticipate and/or render obvious the claimed invention. Indeed, just like the primers of Wilton et al., the primers of Honeyman et al. are designed to ***form*** secondary structures; they are ***not*** designed to, nor will they, cause the disruption of secondary structures, as explained in detail in the Appeal Brief and in this Reply Brief.

Because the claimed invention is not anticipated by Honeyman et al., applicants respectfully request that this Honorable Board order the withdrawal of the anticipation rejection over Honeyman and all of the obviousness rejections that cite Honeyman et al.

C. HOGAL ET AL. DO NOT RENDER THE CLAIMED INVENTION OBVIOUS

The Examiner's arguments in support of the rejection of the claimed invention over Hogan et al. is based upon the Examiner's position that it would be obvious to modify the primers of Hogal et al. to arrive at the claimed invention. Applicants disagree for the reasons set forth in the Appeal Brief and take this opportunity to explain once again why the Examiner's interpretation of Hogan et al. is incorrect.

At page 13 of the Examiner's Answer, the Examiner provides that because the Hogan et al. primer is based upon a target sequence, it would be obvious to arrive at the claimed invention by modifying the probe and helper sequences of the Hogan et al. primer to derive the primers of the claimed invention "since the oligonucleotide probe sequences with folding (hairpin structure formation) taught by Hogan et al. were designed based on the target sequence binding regions, which forms the basis for the primer design."

The Examiner's rationale for modifying Hogan et al. is wrong for at least the following reasons. First, as explained in the Appeal Brief (p. 19), Hogan et al. teach that the helper oligonucleotides disclosed therein ***reorder*** the secondary and tertiary structures of a single stranded nucleic acid to enhance hybridization. Hogan et al. do not teach or suggest the complete disruption of secondary structure in the way of the claimed invention. Second, as also noted in the Appeal Brief (p. 19), Hogan et al. require that the helper oligonucleotides must be in 5-100x excess of the target and/or the probe (Hogan et al. use 100x excess; *see*, col. 8, ll. 36-40); thus, as noted in the Appeal Brief, the Examiner's suggestion that a single sequence of the Hogan et al. helper and probe sequences could be combined with a linking sequence or directly as a

single contiguous sequence does not take into account the 5-100x molar excess of the helper to the target and/or probe. As noted in the Appeal Brief (p. 20), the molar excess of the helper sequence would logically make the sequence so large as to be ineffective. Indeed, it is well-known in primer design that primers tend to be relatively short, typically in the range of 20 nucleotides. Given the requirement of the helper sequence being in 5-100x molar excess of the target and/or probe, it is very unlikely that the single sequence proposed by the Examiner would be of a suitable primer length. In view of the foregoing, applicants submit that contrary to the Examiner's assertion, combining the helper and probe sequence of Hogan et al. is not obvious.

Because the claimed invention is not obvious over Hogan et al., applicants respectfully request that this Honorable Board order the withdrawal of this rejection.

D. CONCLUSION

The foregoing discussion shows that claims 1-18 and 26-35 are not anticipated by or rendered obvious by the cited references. Because the claimed invention is new, useful, and non-obvious, applicants request that the Board reverse the outstanding anticipation and obviousness rejections for this application and allow this case to proceed to issue.

Respectfully submitted,

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